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Effects of the Combination of the Probiotic Probio_Fm and Phytobiotics on the Performance, Gut Dysbiosis, and Lipid Profile of Broiler Meat

Fahmida Manin[1]*, Yusrizal[1], Mairizal[1], Lovita Adriani[2], Andi Mushawwir[2]

[1]Animal Science Faculty, Jambi University, Indonesia; [2]Department of Livestock Nutrition and Feed Technology, Faculty of Animal Husbandry, Universitas Padjadjaran, Indonesia.

Abstract | Probio-FM is a superior probiotic that has been enriched with lactic acid bacteria (LAB) from the digestive tract of geese with a bacterial count of 10^{10}–10^{11} cfu/mL. The purpose of this study was to determine the best combination of probiotics and phytobiotics with improved performance, the ability to reduce the cases of intestinal dysbiosis, lower cholesterol and saturated fat with an increase in unsaturated fat in broiler meat. The treatments were T0 = without Probio_FM and phytobiotics, T1 = Probio_FM + *Curcuma longa*, T2 = Probio_FM + turmeric, T3 = Probio_FM + ginger and T4 = Probio_FM + combined (Curcuma longa, turmeric and ginger). The experimental design used was a completely randomised design with five treatments in four replicates. All variables measured were analysed for variance using analysed using analysis of variance (ANOVA), then if differences were found, it was continued with the DMRT test. The results showed that probiotics and phytobiotics significantly ($P < 0.05$) affected ration consumption, body weight gain, ration conversion, mortality, small intestinal dysbiosis, total cholesterol, triglycerides and blood LDL of broilers, but had no effect on blood HDL levels. It markedly ($P < 0.05$) decreased saturated fat and insignificantly ($P > 0.05$) increased unsaturated fat. This study concluded that small intestine dysbiosis as well as cholesterol, triglyceride, LDL and saturated fat levels in broiler meat.

Keywords | Probiotics, Phytobiotics, Dysbiosis, Lipids

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***Correspondence** | Fahmida Manin, Animal Science Faculty, Jambi University, Indonesia; **Email:** fahmida.manin@unja.ac.id
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INTRODUCTION

Probio_FM is a probiotic solution, containing lactic acid bacteria (LAB), such as *Lactobacillus brevis*, *L. fermentum*, *L. plantarum* and *Pediococcus pentosaceus* (Manin *et al.*, 2014) and also several microbial species isolated from poultry including Kerinci ducks, native chickens reared on peatlands and broiler chickens fed with probiotics. The use of antibiotics is a disadvantageous farming practice. Hence,a need to apply probiotics that are more adaptive to poultry. A study of the validity of this application, especially on gut dysbiosis, as well as performance, is crucial. In addition, it may regulate lipid metabolism so that high growth is not accompanied by increased lipogenicity.

The application of Probio_FM in broilers, laying hens or ducks resulted in improved livestock conditions, decreased livestock mortality and lower ration conversion (Adriani *et*

al., 2021). Ration use conversion was lower in the group that was not given probio_FM than the treatment group, as a result of which, the population of harmful bacteria in the digestive tract of chickens was reduced, so that the villi of the small intestine could better absorb food, reduce the smell of ammonia in faeces (Hendalia *et al.*, 2017; Manin *et al.*, 2010; Manin *et al.*, 2012; Manin *et al.*, 2013, Nurfauziah *et al.*, 2024) and replace antibiotic-based growth promoters.

Previous research attempts have isolated four bacteria namely L. brevis (99.9%), L.fermentum (99.5%), L.plantarum (85.9%) and Pediococcus pentosaecus (99.2%). Furthermore, these four species were combined and grown on MRS broth media; numerous bacteria at 78.3 x 1011 cfu/ml and pH 3.5 were obtained (Manin *et al.*, 2009).

Innovative research was conducted by Manin *et al.* (2008) who transformed liquid Probio_FM into powder by adding prebiotic ingredients of palm kernel cake coconut cakes in a certain ratio (unpublished) to each lactic acid bacteria (L.brevis, L.fermentum, L.plantarum and Pediococcus pentasaceus) and obtained them at log x 11. The results of the research trials by Manin *et al.* (2019) by feeding broiler chickens with Probio_FM powder showed that among the four bacterial species L.fermentum and L.plantarum were able to increase body weight gain (PBB) and reduce Feed Conversion Ratio (FCR), total cholesterol (Dudi *et al.*, 2019) and LDL and LDL cholesterol (Dudi *et al.*, 2019; 2023), but increase blood HDL (Kharazi *et al.*, 2022) and improve the haematologic outcomes (Adriani *et al.*, 2021).

There are indications that the durability of bacteria is highly dependent on the carrier media as a source of nutrients (prebiotics). Several types of carrier media with different shelf lives have been obtained. Bacteria in liquid probio_FM can last for three months at room temperature, while probio_FM powder only lasts for two months at room temperature.

Probio_FM can improve broiler health, by lowering the mortality rate and enhancing the production index (IP). However, farmers still state otherwise. Probiotics do not markedly increase body weight, but at 250 mg in a liquid form improve the intestinal ecosystem (Maksudi *et al.*, 2013; 2014a,b; Manin *et al.*, 2009; Adriani *et al.*, 2021). Therefore, technological innovation is needed to add phytobiotics to Probio_FM probiotics. Some phytobiotics often used are ginger and turmeric (Namagirilakshmi *et al.*, 2010; Tanuwiria and Mushawwir, 2020; Mushawwir *et al.*, 2023). These phytobiotics are expected to stimulate growth (Mushawwir *et al.*, 2024) and prevent excessive fatty meat (Jurenka, 2009; Adriani *et al.*, 2018). Therefore, this combination is very critical in current research.

Phytobiotics are plants that contain chemical compounds that are beneficial to living organisms. The function of phytobiotics in livestock is as an additive in feed formulations to increase livestock productivity (Purwanti *et al.*, 2024). Phytobiotics can be given directly or in the form of extracts and powder preparations (Tanuwiria and Mushawwir, 2020; Tanuwiria *et al.*, 2022) which are mixed in the feed or drinking water.

The functions of phytobiotics include affecting the nervous system (Rahmania *et al.*, 2022), increasing the metabolism (Mushawwir *et al.*, 2021a) and improving body function and immunity (Mushawwir *et al.*, 2021b; Kharazi *et al.*, 2022; Aritonang *et al.*, 2024). The odour and taste produced by phytobiotic plants affect brain function by stimulating the salivary glands and the secretion of digestive juices in the stomach, liver, and pancreas (Adriani *et al.*, 2018) as well as controlling the effectiveness of digestive enzymes in the small intestine (Mushawwir *et al.*, 2022).

Studies combining probio_FM and phytobiotics have not been reported unlike those on the use of each individually. Therefore, this study assesses the impact of the combination of both (probio_FM and phytobiotics) on broilers.

MATERIALS AND METHODS

Probiotic Probio_FM and Phytobiotics Preparation

Probiotics consisting of L. brevis, L. fermentum, L. plantarum and Pediococcus pentosaceus were prepared using 10% of the ingredients. Prebiotics consisting of 1 kg each of palm kernel, soybean and coconut meals were prepared. Phytobiotics consisting of 1 kg each of Curcuma longa, turmeric and ginger were prepared and then shredded. Prebiotics were sterilised in an autoclave, and then cooled to 40°C. Probiotics and each phytobiotic were combined and homogenised. The combination was inoculated with 10% probiotics, incubated for 48 h and then dried at 40°C. After drying, the probiotics and phytobiotics were ready to be given to chickens at a dose of 1% b/v (10g/L drinking water).

Table 1: List of Treatments.

Category	Treatment
T0	without LAB and phytobiotics in drinking water
T1	Probio_FM + curcuma longa
T2	Probio_FM + turmeric
T3	Probio_FM + ginger
T4	Probio_FM + curcuma longa + turmeric + ginger

Note: The dosage used is 10 g each in 1 liter of drinking water (1%).

Birds and Treatments

The animals used for the probio_FM powder + phytobiotic trial were 200 Cobb strain broilers (DOC). The equipment used was one main 6 x 6 m cage. There were 20 unit cages

each of 2 x 1 m. The cages were equipped with feed and drink containers, as well as lamps that function as heaters and for lighting. The feed used was a commercial ration without antibiotics. The design employed was a randomised design with five treatments in four replicates. The treatments are summarised in Table 1. During the rearing period, no vaccination programme was conducted and no antibiotics were supplied to the broilers to enable the optimisation of the treatment effects on the parameters measured.

Broiler Performance Analysis

Ration consumption was measured by recording the amount of ration consumed each day and recapitulating it weekly. Body weight measurement was done by weighing the body weight every week. Ration conversion was calculated by comparing total ration consumption with the final body weight of the week (harvest weight).

Intestinal Dysbiosis Analysis

To measure small intestine dysbiosis in broiler chickens, several parameters were comprehensively evaluated. First, pH was measured by taking a sample of the small intestine, crushing it, mixing it with water in a certain ratio and then measuring the pH with a digital pH metre. Second, the counts of Lactobacillus, E. coli and Salmonella were ascertained. Finally, mortality(%) was calculated by recording the total number of chickens and the number that died during the study, and then using the formula: Number of chickens that died/Total number of chickens x 100%. This measurement can provide a complete overview of gut health and the level of dysbiosis in broiler chickens.

Lipid Level Analysis

Quantitative analysis of saturated and unsaturated fatty acid content was carried out using the Biolabo and Randox KIT protocols following the procedures mentioned (Purwanti *et al*., 2024). The available reagents, containing digestion enzymes, were mixed with a standardised solvent per the instructions and allowed to react with the sample. The intensity of the colour formed was measured using a spectrophotometer.

Statistical Analysis

The data obtained were analysed using analysis of variance (ANOVA). If there was a difference in effects between treatments, then Duncan's multiple range test was performed. Data were analysed by diversity (ANOVA). Data were analysed using the SAS programme 1988 (SAS Institute Inc., North Carolina, USA).

RESULTS AND DISCUSSIONS

Effects of Probio_FM and Phytobiotics on Broiler Performance

The effects of probiotics and phytobiotics powder in drinking water on the amount of feed consumed, body weight gain and FCR are shown in Table 2.

Table 2: Effect of Treatment on Ration Consumption (gr/head/week), Body Weight Gain (gr/head/week) and Ration Use Conversion.

Treatment	Feed consumption (g)	Body weight gain (g)	Feed conversion
T0	477.81±26.68[a]	259.06±14.51[a]	1.73±0.05[a]
T1	420.41±23.48[b]	257.83±13.77[b]	1.61±0.11[b]
T2	407.50±8.50[b]	253.80±10.26[b]	1.58±0.05[b]
T3	393.95±11.50[b]	255.40±7.69[b]	1.54±0.05[b]
T4	406.82±15.14[b]	266.56±7.05[b]	1.58±0.01[b]

Note : Different notations in the same column indicate significant differences (P<0.05) between treatments.

Giving probiotics at 1% or 10 g and a combination of the phytobiotics Curcuma longa, turmeric and ginger at 0.5% or 5 g per litre of drinking water had a significant effect ($P < 0.05$) on ration consumption. Duncan's test results showed that T0 treatment (control) was markedly different ($P < 0.05$) with T1 (probiotics + phytobiotic: temulawak), T2 (probiotics + phytobiotic: turmeric), T3 (probiotics + phytobiotic: ginger) and T4 (probiotics + combined phytobiotics: Curcuma longa, turmeric and ginger). However, there was no variation in ration consumption between T1, T2, T3 and T4. Table 1 shows a significant decrease in feed consumption in T1, T2, T3 and T4 compared to the control, but no effect ($P > 0.05$) on ration consumption in these four.

Less ration consumption was due to the functioning of consisting of L.brevis, L.fermentum, L.plantarum and P.pentasaceus by improving the absorption of food in the small intestines and suppressing the number of pathogenic bacteria in it (competitive exclusion). This can occur because the pH of probiotics is 4.7, while the growth of pathogenic bacteria requires a pH of 6.2–6.8 (Fuller, 1989). The percentage decrease in ration consumption compared to the control treatment (T0) was 12.01% (T1), 19.95% (T2), 20.58% (T3) and 18.02% (T4), with an average of 17.64%, meaning that the provision of probiotics and phytobiotics can save feed by 17.64%.

The results of this study are slightly contrary to the function of phytobiotics, especially Curcuma longa and turmeric which can increase livestock appetite because the phytobiotics contain essential oils. This finding is supported by Adriani *et al*. (2021) who state that the curcuminoids of Curcuma longa play a role in accelerating the secretion of bile in the liver, stimulating the release of pancreatic sap which can increase the metabolism of the feed ingredients, carbohydrates, proteins and fats so that the digestive process takes place quickly and optimally.

The ANOVA results indicated that the treatment of broil-

ers with probiotics and phytobiotics in drinking water had a marked impact (P < 0.05) on body weight gain. Further, Duncan's test showed that T0 was significantly different (P < 0.05) from T1, T2, T3 and T4, but there was no significant difference (P > 0.05) between the T1, T2, T3 and T4 on body weight gain. The decrease in body weights were T1 = 4.33%, T2 = 6.16%, T3 = 5.09% and T4 = 5.09 compared to T0 with an average of 5.30%. The decrease in body weight was closely related to the reduction in ration consumption ranging from 12.01%–20.58%.

Feed conversion is one of the indicators of business success for farmers. It is obtained from the ratio of the amount of feed consumed to body weight gain in a specific time. The ANOVA results indicated that treatment with probiotics and phytobiotics had a marked impact (P < 0.05) on feed conversion. Further, using the Duncan test revealed that the provision of probiotics and phytobiotics in drinking water can reduce ration conversion compared to the control. The reduction in feed conversion was 28.40% (T1), 48.76% (T2), 27.27% (P-3) and 15.11% (P-4), with an average of 29.98%. This decrease was due to the reduction in ration consumption followed by a decline in body weight gain. From Table 1, it can be observed that the feed consumption at T0 was higher than that at T1, T2, T3 and T4. It was followed by higher body weight gain in the control treatment resulting in high FCR. Based on these data, it can be suggested that the role of beneficial microbes in the digestive tract is very decisive, although they are naturally present in the digestive tract.

Gut Dysbiosis

Small gut dysbiosis is a condition in which the number of pathogenic bacteria outnumber the non-pathogenic ones in the small intestine. Pathogenic bacteria in poultry include E.coli and Salmonella, while the non-pathogenic bacteria were generally the LAB, Lactobacillus. The results of the small gut dysbiosis study are presented in Table 3.

Table 3: Mean pH, number of Lactobacillus, E.coli, salmonella and Mortality (%).

Treatments	pH	Bacteria Count (transformation Log x)			Mortality	
	Value	Lactobacillus	E.coli	Salmonella	Total	%
PO	6.11[a]	10.7650[a]	8.3168[a]	1.6176[a]	6[a]	15[a]
P1	5.78[b]	11.5189[b]	7.4328[b]	0[b]	1[b]	2.5[b]
P2	5.88[b]	11.3258[b]	7.7104[b]	0[b]	0[b]	0[b]
P3	5.82[b]	11.3833[b]	7.7408[b]	0[b]	0[b]	0[b]
P4	5.81[b]	11.3800[b]	7.7792[b]	0[b]	0[b]	0[b]

Note : Different notations in the same column indicate significant differences (P<0.05) between treatments.

The ANOVA results revealed that the treatment of broilers with probiotics and phytobiotics in drinking water had a significant effect (P < 0.05) on the acidity (pH), the number of Lactobacillus, E.coli and Salmonella, in the small intestine and mortality. Further, Duncan's test found that T0 varied markedly (P < 0.05) with T1, T2, T3 and T4, but not between the four treatments (P > 0.05).

The acidity (pH) value post-treatment without probiotics and phytobiotics was 6.11 and was higher than with treatment with T1, T2, T3 and T4. However, there was no statistical difference between the probiotic + phytobiotic treatments. The decrease in acidity (pH) in T1, T2, T3 and T4 was due to the addition of 10 g of each probiotic in 1 L of drinking water. The probiotics contain Lactobacillus brevis, L. fermentum, L. plantarum and Pediococcus pentasaceus at 53 x 1010 cfu/g and a pH value of 4.5; with the addition of these LAB, the pH of the small intestine dropped from 6.11 to 5.78 (T1), 5.88 (T2), 5.87 (T3) and 5.81 (T4).

This low acidity value causes the number of LAB (Lactobacillus) to be more than the treatment without probiotics and phytobotics. One of the functions of probiotics is to reduce the number of pathogenic bacteria, E.coli and Salmonella in the small intestine of chickens through the mechanism of 'competitive exclusion', namely through competition for living space within the small intestine. The number of Lactobacillus bacteria were, in T0: 10.7650, 11.5189: T1, 11.3258: T2, 11.3833: T3 and 11.3800: T4 (log x transformation) per gram of small intestine contents.

The increase in Lactobacillus can reduce E.coli in T1, T2, T3 and T4, over T0. Likewise, Salmonella in T0 was found at 1.6176 (log x transformation). However, in T1, T2, T3 and T4, Salmonella was not detected. The increase in the number of E.coli and Salmonella in T0 caused high mortality (six heads or 15%) while in T1 treatment it was one head or 2.5%. These results indicate that combined probiotics and phytobiotics play an excellent role in preventing the growth and development of pathogens. The effectivity of probiotics as appreciable anti-pathogen results from the active peptides contained by LAB. Research conducted by Adriani *et al.* (2021) and Jin *et al.* (2006) revealed the active peptide contents and their effect on eliminating pathogenic bacteria. Combination with phytobiotics enhances the efficacy of this additive. Ginger and temulawak contain curcumin, flavonoids and essential oils (Mushawwir *et al.*, 2023). Flavonoids and curcumin lyse the cell membranes of pathogenic bacteria (Mushawwwir *et al.*, 2021; Rahmania *et al.*, 2022), prevent the metabolism and replication of pathogenic bacteria (Zhang *et al.*, 2009; Mushawwir *et al.*, 2022, 2024; Jin *et al.*, 2006).

Lipids

The results of this study showed that the average saturated and unsaturated fatty acids were T0 (34.29%: 58.83%), T1 (29.83%: 58.25%), T2 (27.74%: 55.12%), T3 (29.45%:

56.89%) and T4 (31.30%: 60.75%) (Table 4). Based on the ANOVA results, the treatment had a marked impact ($P < 0.05$) on the percentage of unsaturated fats, but an insignificant effect ($P > 0.05$) on that of unsaturated fats. The highest reduction in saturated fat was in T2 at 19.10%, T3 (14.11%), T1 (13.00%) and T4 (8.72%). This observation was due to several types of saturated fatty acids not being detected or not present in chicken meat.

Table 4: Average percentage of meat fat and cholesterol of broiler meat.

Treatment	Fat	Cholesterol	Fatty Acid	
	%	%	Saturated (%)	Unsaturated (%)
T0	2.57[a]	18.20[a]	34.29[a]	58.83[a]
T1	2.83[a]	18.21[a]	29.83[b]	58.25[a]
T2	2.13[b]	18.81[a]	27.74[b]	55.12[b]
T3	2.43[b]	19.86[a]	29.45[b]	56.80[b]
T4	2.43[b]	17.71[b]	31.30[b]	60.75[c]

Note : Different notations in the same column indicate significant differences ($P<0.05$) between treatments.

Administration of Probiotic_FM with a combination of phytobiotics enhanced the liposis rate so that energy reserves in the form of fat would be broken down into NEFA and glycerol. NEFA is also a precursor for the formation of triglycerides (TAG) in the adipose tissue, liver and muscle through esterification and is stored in the form of meat and abdominal fat (Mushawwir *et al*., 2023; Adriani *et al*., 2018). In contrast, the triglyceride profile without irradiated chitosan and with glutathione induction was higher than the NEFA profile of broiler chickens treated with the two.

However, in this study, it seems that the treatment with probiotics and phytobiotics does not stimulate the formation of saturated fat, because they can reduce the activity of lipoprotein lipase which is a hydrolyser, oxidising chylomicrons and free fatty acids (NEFA) into triglycerides (Mushawwir *et al*., 2022). The synthesis of triglycerides in the exogenous pathway, namely those derived from food in the intestine are packaged as chylomicrons which are then transported in the blood through the ductus torasikus; triglycerides and chylomicrons in fatty tissue undergo hydrolysis catalysed by lipoprotein lipase present on the surface of endothelial cells to form fatty acids and chylomicrons (Kharazi *et al*., 2022).

This metabolic phenomenon does not encourage the formation of saturated fats, but instead enhances the synthesis of unsaturated fatty acids (Adriani *et al*., 2018). Free fatty acids enter fat tissue or muscle cells by penetrating the endothelium and are then re-oxidised or converted into double long-chain fatty acids (Mushawwir *et al*., 2024; Rahmania *et al*., 2022). The active compounds in Curcuma longa, turmeric and ginger played a crucial role in reducing lipid concentrations in this study. Mushawwir *et al*. (2021a) showed the ability of ginger flavonoids to inhibit lipid synthesis. The activation of lipid regulatory genes causes lipid homeostasis to be maintained. Another study showed the inhibition of *AMPK* after the administration of turmeric containing curcumin (Mushawwir *et al*., 2022; Purwanti *et al*., 2024). Flavonoids also prevent excessive activation of HMG-CoA reductase during cholesterol biosynthesis (Mushawwir *et al*., 2024; Namagirilakshmi *et al*., 2010; Kharazi *et al*., 2022).

CONCLUSIONS AND RECOMMENDATIONS

The results of this study show that the Probiotic_FM and phytobiotic combination (T4) was the best treatment. Both can reduce FCR, mortality, total cholesterol, triglycerides and LDLs, but are unable to increase blood HDL. Both reduce small gut dysbiosis (decrease in small gut pH, reduce the number of E.coli and Salmonella but increase the number of LAB). Both can reduce saturated fat but are unable to enhance unsaturated fats in broiler meat. Such research needs to be continued by increasing the concentration of probiotics and phytobiotics and determining the cholesterol and unsaturated fatty acids in meat.

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NOVELTY STATEMENT

The use of the Probiotic_FM and a combination of phytobiotics is the latest breakthrough in the research and development of feed additives in Indonesia. Global independent research for these two feed additives individually exists, but the use of both (probitok_FM with phytobiotics) has never been studied and their interaction in the broiler metabolic system determined.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the writing of this manuscript.

Ethical Approval

All procedures of this study have been reviewed and approved by the Ethical Review Committee for Animal Ex-

periments, Directorate of Research and Technology, DRT (Number: 2721/An.Res.ER/07/24).

Conflicts of Interest

The authors have no conflicts of interest to declare.

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